Supporting Appendix

DNA Damage Repair. An ORF for the Ada regulatory protein, which mediates repair of alkylation damage, is present on each chromosome. The Ada proteins have a regulatory domain together with a domain for *O*-6-methyl-DNA-alkyltransferase activity. Two additional ORFs encode *O*-6-methyl DNA alkyltransferase activity; one similar to Ogt and the other similar to AlkA in the base excision repair pathway. DNA ligation is mediated by an NAD-dependent DNA ligase. The absence of a DNA photolyase suggests that UV-induced damage is repaired via "dark repair." Nucleotide excision repair is mediated by the UvrABCD complex and also uses transcription-coupled repair (TCR), using protein Mfd**.** The MutT (8-oxo-dGTPase) enzyme removes the 8-oxoG-modified bases that result from oxidative damage. Two copies of *xthA*, encoding the major AP nuclease ExoIII, are present. No ORF for uracil DNA glycosylase has been identified. Genes for MutSL and DNA helicase II are present, but the essential ORF for MutH and repair endonuclease (Vsr) were not identified. The recombination repair system proteins RecA and RecJ, the Holliday junction resolvase complex RuvABC, and the SOS/error prone pathways are present.

A single type I restriction/modification system has been identified. It consists of an operon containing four ORFs, one for the S subunit of the enzyme, followed by a DNA methylase, an EcoR1 24II specificity protein, and restriction enzyme HsdR.

Stress Response Genes. Heat-shock proteins such as DnaJ, two copies of DnaK, GroEL, GroES, and three cold-shock proteins (CspA) are present. ORFs for stress-induced proteins such as ClpA, ClpB, ClpP, and ClpX are located on chromosome I. ClpB has been shown to be required for protein degradation during acid, ethanol, and high-temperature stress response in *B. suis* (1). Two ORFs on chromosome I and one on chromosome II encode stress-induced proteins similar to the UspA of *E. coli* that is induced under a variety of stress conditions such as heat shock, carbon starvation, and osmotic shock.

Transport. *B. melitensis* also contains several secondary carbohydrate transporters. The glucose/galactose transporter GluP, identified in *B. abortus* (2), is also found in *B. melitensis.* Several additional major facilitator superfamily (MFS)

transporters with similarity to carbohydrate transporters are present, although their substrates cannot be predicted with a high degree of confidence. The phospho*enol*pyruvate sugar phosphotransferase (PTS) system does not seem to be used for carbohydrate transport in *B. melitensis* but may be utilized for signal transduction. The PTS system contains one copy of enzyme I, IIA, and HPr. ORFs for the enzyme IIB subunit and the integral membrane protein of the PTS system are absent. PTS homologs present are similar to the *ptsN*, *npr*, and *ptsP* gene products that are involved in nitrogen regulation rather than transport in *E. coli* (3).

B. melitensis encodes 10 transporters that belong to the ABC transporter families, of which seven import amino acids. One is similar to the general amino acid transporter of *Rhizobium*, which has broad uptake specificity (4). Seven are related to branched-chain amino acid transporters. Four additional amino acid-binding proteins that are not part of the ABC transporter operon but are involved in amino acid uptake are found. There is one ORF that encodes a protein similar to a Glu/Gln/Asp/Asn- family binding protein, one arginine-binding protein, and two related to the arginine-ornithine binding proteins. One of the arginine-ornithine binding protein genes is truncated and may not be functional. There are two members of the amino acid permease family; one has high similarity to a D-serine/D-alanine/glycine:proton symporter that is found in *E. coli*, whereas the other is related to amino acid antiporters involved in acid resistance. In addition, a member of the sodium:alanine symporter family and an ammonium transporter are present. Adjacent to the gene encoding urease is an ORF that encodes a protein similar to the eukaryotic urea transporter. There is also an ORF with weak homology to the *E. coli* cyanate transporter.

Genes that encode specific transporters for ion homeostasis by accumulation of potassium or magnesium and exclusion of sodium, respectively, were observed. No calcium exporter seems to be present. There is also a *trk* potassium uptake system that codes for the TrkA and TrkH proteins and a homolog of the *kup* operon for the potassium uptake transporter. Several putative magnesium transporters such as MgtE, CorA, and a P-type ATPase similar to the *Salmonella* MgtB were found. The MgtB homolog has been shown to be important for *B. melitensis* virulence (5). Sodium/proton antiporters often export sodium; in *B. melitensis*, there is a protein with significant homology to the

NhaA-type sodium/proton antiporter. The *B. melitensis* NhaA is an unusual protein as it seems to be fused to a protease. Potassium/proton antiporters are involved in adaptation to changes in pH or in the response to certain toxins. *B. melitensis* has an operon that is similar to *phaABCDEFG* found in *S. meliloti.* The *phaABCDEFG* operon encodes a potassium/proton antiporter that responds to the changes of pH during interaction with the host (6). In addition, there are two proteins similar to a glutathione-regulated potassium/proton antiporter (7).

B. melitensis lacks a ferrous iron transporter but can transport ferric iron-siderophore complexes across the outer membrane through receptors energized by the TonB/ExbB/ExbD protein complex. The ExbB and ExbD proteins are involved in biopolymer transport with TonB. The genome contains three TonB-dependent receptors, two of which seem to be specific for siderophores and one for copper. A nickel ABC transporter similar to the one characterized in *B. suis* is present (8). There are individual putative ABC transporters for uptake of cobalt, molybdate, and zinc, and four ABC transporters involved in uptake of iron or chelated iron siderophores. There is also an ORF for a probable ABC transporter for copper uptake, but the ATP-binding protein of this putative copper transporter is absent.

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